Photolysis of Bovine Serum Albumin by near UV irradiation

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Sarah Foley[1], Angela Staicu[2], Alexandru Pascu[2] & Mironel Enescu[1]

1. LCPR, UMR CEA E4, 16 route de Gray, Université de Franche-Comté, 25030 Besançon, France
2. National Institute for Lasers, Plasma,and Radiation Physics, 409 Atomistilor Str., Magurele, Ilfov, 077125 Romania

Introduction

Bovine Serum Albumin (BSA) is the most abundant protein in blood plasma and serves a depot as well as a transport protein for numerous compounds. The structure of BSA is for the most part helical (70%). BSA is also a protein characterised by its high pecentage of lysine residues (10 % of its amino acid content). It is well known that side chains containing Tyrosine and Tryptophan play a dominant role in the near UV electronic absorption and subsequent fluorescence of proteins. However, proteins rich in lysine residues possess an unusual spectral absorption tail between 300 – 400 nm [1]. This band can play an important role in understanding protein damage induced by near UV irradiation.

We report here the photolysis of BSA due to irradiation at 355 nm and studied using Raman spectroscopy which allows the spectral changes induced by photolysis to be directly identified.

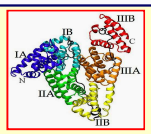


Fig. 1. Ribbon diagram of BSA

Methods

Raman Measurements:

Raman spectra for all solutions were measured in the region of 380 – 1750 cm^{-1} using the second harmonic of an Nd/YAG laser ➔ 532 nm with a pulse duration of 5 ns.
Solutions were irradiated using 355 nm with a fluence of 10 mW / mm^2 for 30 or 60 minutes.
All solutions were measured at pH = 7 using a concentration of 1 mM BSA and the reference solution used throughout the measurements was H_2O.
The wavenumbers of Raman bands were reproducible within ±1 cm^{-1}.

Emission Spectra:

Fluorescence measurements were made using a Horiba Jobin Yvon Fluorolog®-3-22 flurometer.

The instrument settings were as follows:
Emission slits = 5.0 nm
Excitation slits = 5.0 nm

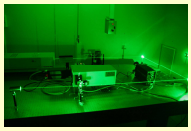


Fig. 2. Experimental set up for Raman Spectroscopy

Results & Discussion

I. Excitation and Emission Spectra

Fig. 3 shows the emission spectra obtained when a solution of BSA is excited at 400 nm. Emission shows a maxima at 463 nm which is slightly red shifted and increases in intensity after irradiation.

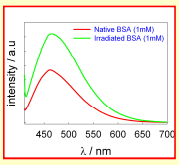
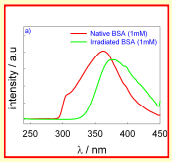


Fig. 3. Emission spectra of native BSA and photolysed BSA, λ_{ex} = 400 nm.



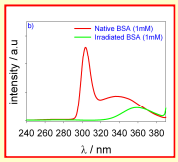


Fig. 4. Excitation spectra of native BSA and irradiated BSA:
a) λ_{em} = 460 nm, b) λ_{em} = 400 nm

Fig. 4 shows the excitation spectra of BSA and highlights two species with maxima situated at 360 nm and 306 nm. Following irradiation, the peak at 360 nm is red shifted and decreases in intensity whilst the peak at 306 disappears, thus indicating the involvement of both species in the photolysis of BSA.

II. Raman Spectra

The Raman spectrum for native BSA between 380 and 1750 cm^{-1} is presented in figure 5a.
Figures 5b and 5c show the Raman spectra of BSA after irradiation with 355 nm light under aerobic and anaerobic conditions.

The absence of any changes between spectra 5b and 5c suggests that the changes observed following the photolysis of BSA are not mediated by singlet molecular oxygen.

Changes are observed in three regions of the spectra :
1 - Between 480 – 600 cm^{-1} (S-S band)
2 - Between 1200 – 1400 cm^{-1}
3 - Between 1520 – 1700 cm^{-1} (Amide I band)

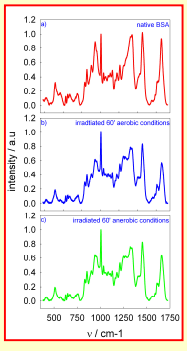


Fig. 5. Raman spectra of BSA and irraditaed BSA under aerobic and anerobic conditions.

III. Structural Modifications of the Protein

The irradiation of BSA brought about some structural changes which manifested themselves in a number of ways.

Changes in the S-S band

Photolysis of BSA results in significant changes in the Raman S-S band.
During the reaction the intensity of the band falls with components 2 and 3 showing a marked decrease (Figure 6).
The evolution of the S-S bands under irradiation strongly suggests that the S-S bridges are partially reduced.

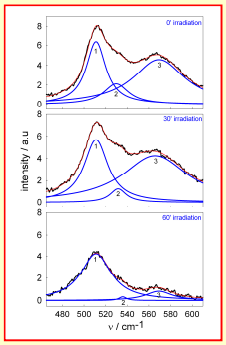


Fig. 6. Analysis of the S-S Raman band for 1 mM BSA as a function of irradiation time.

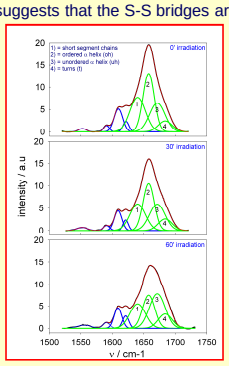


Fig. 7. Analysis of the Amide I Raman band for 1 mM BSA as a function of irradiation time.

Irradiation time / min	Peak 1	Peak 2	Peak 3	Peak 4
0	29.84	36.77	24.96	8.43
30	27.19	34.95	27.17	10.69
60	24.84	26.50	35.69	12.97

Table 1. Analysis of the Amide I band showing the % of each peak as a function of irradiation time

Changes in the Amide I band

Analysis of the Raman band provides information about the protein secondary structure and can be analysed using the following attribution [2].
Peak 1 at 1640 cm^{-1} – short segment chains
Peak 2 at 1657 cm^{-1} – the ordered α-helix
Peak 3 at 1671 cm^{-1} – the unordered α-helix
Peak 4 at 1684 cm^{-1} – vibrations of various turns

Following the photolysis of BSA after irradiation at 355 nm the cleavage of the disulphide bridges is associated with a significant evolution of the Amide I band (Table 1 and Figure 7).
Changes in the secondary structure suggesting protein unfolding can be summarised as:
↘ decrease in the fraction of the ordred α-helix
↗ increase in the fraction of the unordered α-helix, protein unfolding.

Fluorescence Measurements

The photolysis of BSA was also followed via the fluorescence spectra of the tryptophan residues.
- red shift in the intrinsic tryptophan fluorescence of BSA
- ↘ in the intensity of emission

Indicative of a change in the tertiary structure of the protein.

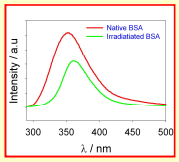


Fig. 8. Emission spectra of native BSA and irradiated BSA, λ_{ex} = 400 nm.

Mechanism

The mechanism for the photolysis of BSA at 355 nm is at present unknown, however one can consider the following :

$$Chr \xrightarrow{h\nu(355nm)} Chr^{*+} + e^-$$

$$RSSR + e^- \rightarrow RS^{\bullet} + RS^-$$

Whereby Chr is the chromophore absorbing at 355 nm and by which the reduction of the disulphide bridges results in the unfolding of the protein as previously described [3].

Conclusions

The photolysis of BSA at 355 nm is followed by a partial reduction fo the disulphide bridges.
Disulphide bridge reduction gives rise to a modification of the secondary structure of the protein due to unfolding.
The photolysis of BSA also brings about changes in the tertiary structure of the protein.

References

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